APPENDIX B
PENDING CLAIMS SUBJECT TO EXAMINATION

1. (Twice amended) A method of counting a single copy of a target species immobilized on a substrate, said method comprising:

(i) detecting a single copy of said target species by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, and further wherein said first quantum dot is distinguishable from said second quantum dot.

2. (Once amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are attached to said target species prior to binding said target species to said affinity moiety.

3. (Once amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are attached to said target species after binding said target species to said affinity moiety.

5. (Once amended) The method according to claim 1, wherein binding of said target species to said affinity moiety forms a target species-affinity moiety complex that is detected by fluorescence from both said first quantum dot and said second quantum dot attached to said target species-affinity moiety complex.

6. (Once amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are distinguishable by a characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

7. (Once amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are visually distinguishable as a first color and a second color, respectively.

8. The method according to claim 7, wherein said first color and said second color combine to form a visually or electronically distinguishable color different from both said first color and said second color.

9. The method according to claim 1, wherein said target species has *n* quantum dots attached thereto, wherein each of said *n* quantum dots is distinguishable from each other, and *n* is an integer from 3 to 10.

10. (Twice amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are attached to a targeting moiety for said target species, said targeting moiety being a member selected from the group consisting of antibodies, aptamers, proteins, streptavidin, nucleic acids and biotin.

11. The method according to claim 1, wherein said affinity moiety is labeled with a quantum dot.

12. The method according to claim 1, wherein said target species is a member selected from the group consisting of organisms, biomolecules and bioactive molecules.

13. The method according to claim 1, wherein said affinity moiety is a member selected from the group consisting of organisms, biomolecules and bioactive molecules.

14. The method according to claim 1, wherein said substrate has bound thereto a second affinity moiety.

15. The method according to claim 14, wherein said first affinity moiety and said second affinity moiety are different affinity moieties.

16. The method according to claim 1, wherein said substrate has bound thereto *m* affinity moieties; and *m* is an integer from 1 to 10,000.

17. The method according to claim 16, wherein each of said *m* affinity moieties is a different affinity moiety.

18. The method according to claim 16, wherein said *m* affinity moieties are ordered in an array format.

19. The method according to claim 1, wherein said substrate further comprises an alignment moiety providing a reference point on said substrate for the detection of a target-affinity moiety complex formed between said target and said affinity moiety, wherein said target-affinity moiety complex is distributed upon said substrate in a random manner, said alignment moiety comprising a fluorescent label, which does not interact with said target species or said affinity moiety.

20. The method according to claim 19, wherein said alignment moiety comprises a quantum dot.

21. The method according to claim 19, wherein said alignment moiety is distinguishable from each quantum dot attached to said target species.

22. (Twice amended) The method according to claim 19, wherein said alignment moiety identifies the position of one or more target moiety-affinity complexes.

23. The method according to claim 1, wherein said substrate is manufactured from a low fluorescence optical material configured as a member selected from the group consisting of a microtiter plate, a glass slide, a microscope slide cover slip, a capillary, a flow cell, a bead and combinations thereof.

24. The method according to claim 1, further comprising, counting each detected quantum dot per unit area of said substrate, producing substrate quantum dot data; and comparing said substrate quantum dot data with standard quantum dot quantity data

acquired from a standard of said quantum dot having a known concentration, thereby quantifying said target species immobilized on said substrate.

25. (Once amended) A computer-readable medium encoded with a data set comprising data acquired by the method of claim 1.

28. (Once amended) A computer-readable medium encoded with a database comprising two or more data sets according to claim 25, wherein said database is in a searchable format.

29. (Twice amended) A method of counting a single copy of a target species in solution, said method comprising

(i) detecting a single copy of said target species by detecting essentially simultaneously an optical characteristic of a first quantum dot of a first color attached to said single copy and a second quantum dot of a second color attached to said single copy, wherein said first color and said second color are distinguishably different colors, thereby counting said single copy.

30. (Twice amended) A method of counting a single copy of a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate with spacing between each member of said population, said method comprising:

(i) detecting a single copy of said target species by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, wherein said first quantum dot is distinguishable from said second quantum dot, and further wherein said detecting is performed with a detecting means having a resolution that is higher than said spacing between each member of said population, thereby counting said single copy.

31. (Twice amended) A method of counting a single copy of a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

(i) detecting a single copy of said target species by detecting an optical characteristic of a quantum dot attached to said single copy, wherein said first quantum dot is distinguishable from said second quantum dot, and further wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate forming a target-affinity moiety complex, and said detecting is performed with a detecting means having a resolution limited region of interest whereby, less than one target-affinity moiety complex is present within each resolution limited region of interest, thereby counting said single copy.

32. (Twice amended) A method of counting a single copy of a first target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

(a) defining a first region of interest of said substrate; and

(b) probing said first region of interest for an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said first target species bound to an affinity moiety for said first target species immobilized on said substrate, wherein said first quantum dot is distinguishable from said second quantum dot, thereby counting said first target species.

33. (Twice amended) The method according to claim 32, further comprising counting a single copy of a second target species immobilized to said substrate, said method comprising:

(c) defining a second region of interest of said substrate; and

(d) probing said second region of interest for an optical characteristic of a third quantum dot and a fourth quantum dot attached to said single copy of said second target species bound to an affinity moiety for said second target species immobilized on said substrate, wherein said third quantum dot is distinguishable from said fourth quantum dot, thereby counting said second target species.

34. The method according to claim 33, wherein said first region of interest and said second region of interest are the same region of interest.

35. The method according to claim 32, wherein said probing is by a method selected from the group consisting of microscopy, confocal fluorescence microscopy and two-dimensional imaging with a CCD camera.

36. The method according to claim 32, wherein said first target species and said second target species are different species.

37. (Once amended) A method for counting multiple target species immobilized on a substrate, which species are members of a population of target species immobilized on said substrate, said method comprising:

(a) defining multiple regions of interest on said substrate; and

(b) probing said multiple regions of interest for an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of said target species bound to an affinity moiety for said target species immobilized within a region of interest of said substrate, thereby counting multiple target species.

38. (Once amended) A method for determining whether a target species within a region of interest on a substrate is quantifiable by a technique selected from the group consisting of single target counting and ensemble counting, said method comprising:

(a) probing said region of interest to determine target species density within said region of interest by detecting fluorescence emitted by a quantum dot attached to one or more molecules of said target species bound to an affinity moiety for said target species immobilized on said substrate;

(b) comparing said density to a predetermined density cutoff value above which ensemble counting is used and below which single target counting is used, thereby determining whether said target species is quantifiable by target counting or ensemble counting.

39. The method according to claim 38, wherein said substrate comprises a first region in which ensemble counting is used and a second region in which single target counting is used.

40. (New) The method according to claim 1, wherein said optical characteristic is detected by coincidence detection.

41. (New) The method according to claim 1, wherein said optical characteristic is fluorescence.

42. (New) The method according to claim 29, wherein said optical characteristic is fluorescence.

43. (New) The method according to claim 31, wherein said optical characteristic is fluorescence.

44. (New) The method according to claim 32, wherein said optical characteristic is fluorescence.

45. (New) The method according to claim 33, wherein said optical characteristic is fluorescence.

46. (New) The method according to claim 37, wherein said optical characteristic is fluorescence.

47. (New) The method according to claim 1, further comprising

(ii) resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy from an optical characteristic arising from a quantum dot not attached to said single copy.

48. (New) The method according to claim 29, further comprising

(ii) resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy from an optical characteristic arising from a quantum dot not attached to said single copy.

49. (New) The method according to claim 32, further wherein said probing resolves said optical characteristic of said first quantum dot and said second quantum dot from an optical characteristic of other members of said population of target species immobilized on said substrate.

50. (New) The method according to claim 33, further wherein said probing resolves said optical characteristic of said third quantum dot and said fourth quantum dot from an optical characteristic of other members of said population of target species immobilized on said substrate.

51. (New) The method according to claim 37, wherein said probing resolves the optical characteristic of said first quantum dot and said second quantum dot from other members of said population and from each other.